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Somatic Effects of low-intensity Radiation at different Levels of biological Organization

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In the study of the effects of low-intensity radiation, one is handicapped by the difficulty of designing quantitative methods for the detection of the effects. Inability to recognize the damage to individual cells in the presence of a very large number of cells that are effected little or not at all by radiation is often an insurmountable handicap. One also has to face the fact that this individual cell damaged by radiation might be the one that is the origin of some malformation and possible source of a malignancy. In microorganisms this difficulty was overcome by eliminating all cells that had certain nutritional requirements not present in the growth medium. It is not possible to use this technique with organized tissues; however, the new type of culture technique developed for mammalian tissue may change this. On the basis of a few fundamental studies, I will discuss the direction in which work of this type is developing. I will also emphasize in the discussion that a high percentage of the damage is reversible and that it has been possible to protect against a good part of the detrimental effects of radiation by a variety of procedures.

First let us deal with the rate of mitosis (Fig. 1), a basic reaction present in all living cells, and a reaction that lends itself to a well-defined analysis. This will be illustrated on one tissue that has been investigated most thoroughly (*Carlson*, 1954) the grasshopper neuroblast, where the effect of as little as 1 r can be readily recognized. Similar observations have been made on the skin of the ear of the mouse and could probably be made on many other tissues. If one gives a small amount of X or ultraviolet radiation to these cells, the regular mitotic rate will be upset and cells will go into prophase and wait there for a

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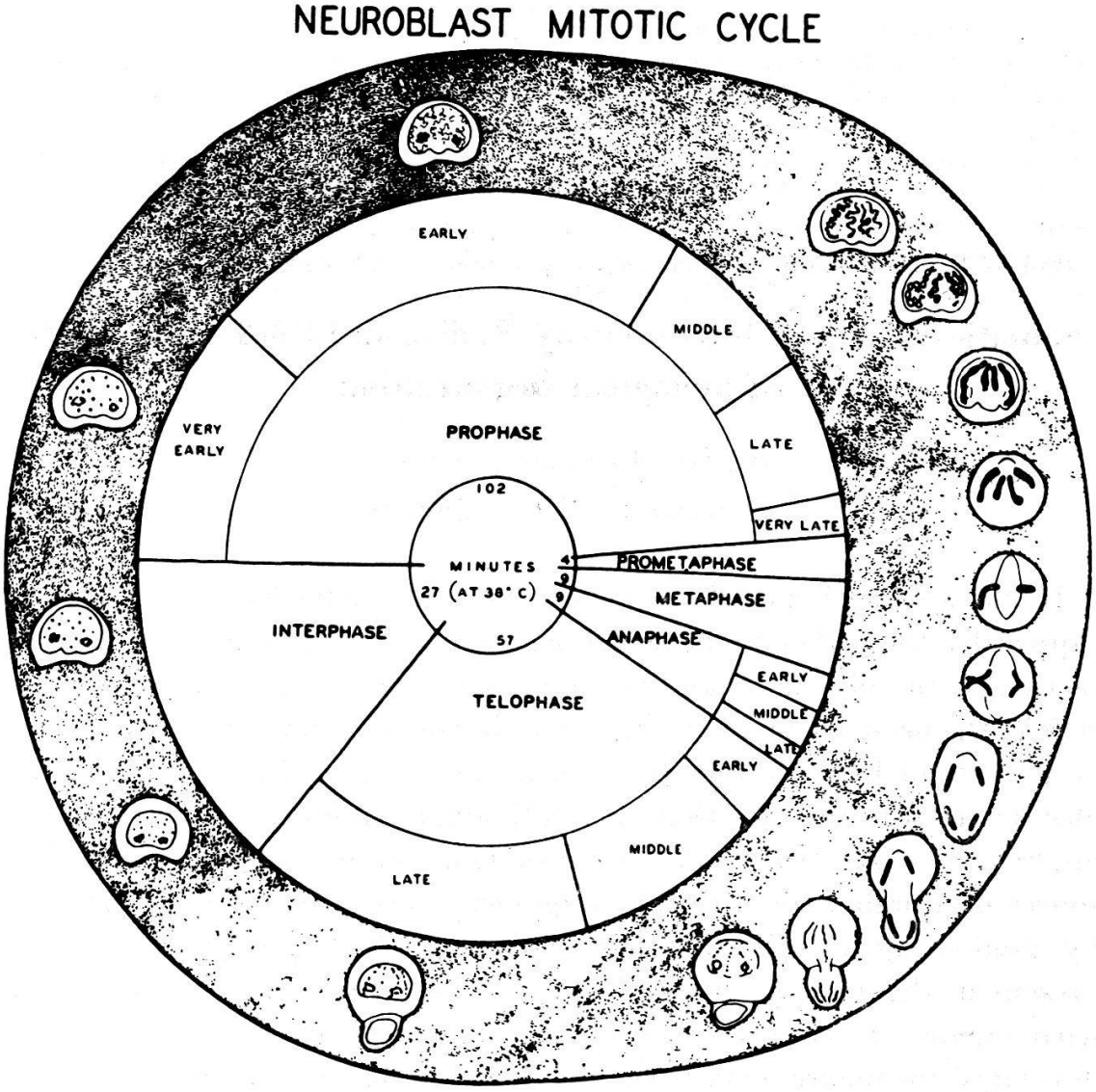

Fig. 1. Mitotic cycle in the grasshopper neuroblast (from *Carlson* and *Hollaender*, 1948).

considerable time before resuming their routine mitotic steps. As a matter of fact, at certain stages of mitosis the mitotic rate can be reversed. It has been shown, as you probably know, that oxygen has a definite effect on X-ray sensitivity of most living tissues. In the absence of oxygen, the cells are much more resistant. In the case of the rate of mitosis, if the cells are irradiated in the absence of oxygen they remain in the delayed, retarded phase a much shorter time and they return to normal more rapidly. Another way of counteracting radiation damage has been found lately by Dr. *M. E. Gaulden* of our laboratory. It has been shown that if one uses a slightly hypertonic salt solution on these cells, the rate of mitosis will be speeded up to a small degree. If one uses hypertonic salt solution immediately after irradiation, i.e., within sixty seconds, all the radiation damage up to 30–40 r can be prevented. This is an interesting observation because it shows that the

effects of radiation usually observed a considerable time after exposure may be a late step in a number of reactions that finally lead to the damage that we recognize. The immediate effect of radiation may be nothing but an upset of the ionic concentration in the different parts of the cell. In any case, investigation of the rate of mitosis has shown some new possibilities for counteracting radiation damage.

Next I would like to mention the influence of radiation on chromosome breaks since these chromosome breaks may have serious genetic implications. They may produce dominant genetic effects, and often lead to the death of the cell. Since the genetic effects will be discussed by Dr. *Fritz-Niggli*, one of the speakers to appear later on this program, I will omit a detailed discussion on this point. However, it should be pointed out that it is now possible to help the cell repair some of the chromosome breaks in a number of tissues by treatment after exposure by supplying an energy source (*Wolff*, 1957).

Now we come to a discussion of radiation effects on spermatogonia. Most of this work has been done with mice and rats, but, in general, the results can be applied to all animals. Basic techniques have been developed in laboratories in Canada and in Europe, but a considerable amount of this work has been done in the Biology Division of the Oak Ridge National Laboratory. (For a review of earlier basic studies see *Schinz* and *Slotopolsky*, 1925.) A good part of this discussion will be based on the work of Dr. *E. F. Oakberg* (1955, 1956). Some stages in the development of the sperm are extremely sensitive to the effects of radiation. You probably are quite well acquainted with the general radiation response, i.e., if a male is exposed to significant amounts of radiation (but not over 1000 r), fertility is retained for a few weeks; then a temporary sterile period occurs, the length of which is dependent on dose; and finally almost a normal level of fertility is regained. The sperm available immediately after exposure may show dominant effects, particularly death of embryos *in utero* and partial fertility of the surviving progeny. Sperm used after the sterile period, i.e., gametes developed from cells irradiated as spermatogonia, will not show an increase in dominant effects but will show the effects resulting from gene mutation, which will be discussed later on.

Since the mature sperm is highly resistant to radiation—it requires thousands of roentgens to impair the ability to fertilize an ovum—it is interesting that certain spermatogonia, the early stages in spermatogenesis, are extremely sensitive to radiation. In order to interpret the data I wish to present, a brief description of spermatogenesis is necessary. Each dormant type A (dusty) spermatogonium (stem cell) under-

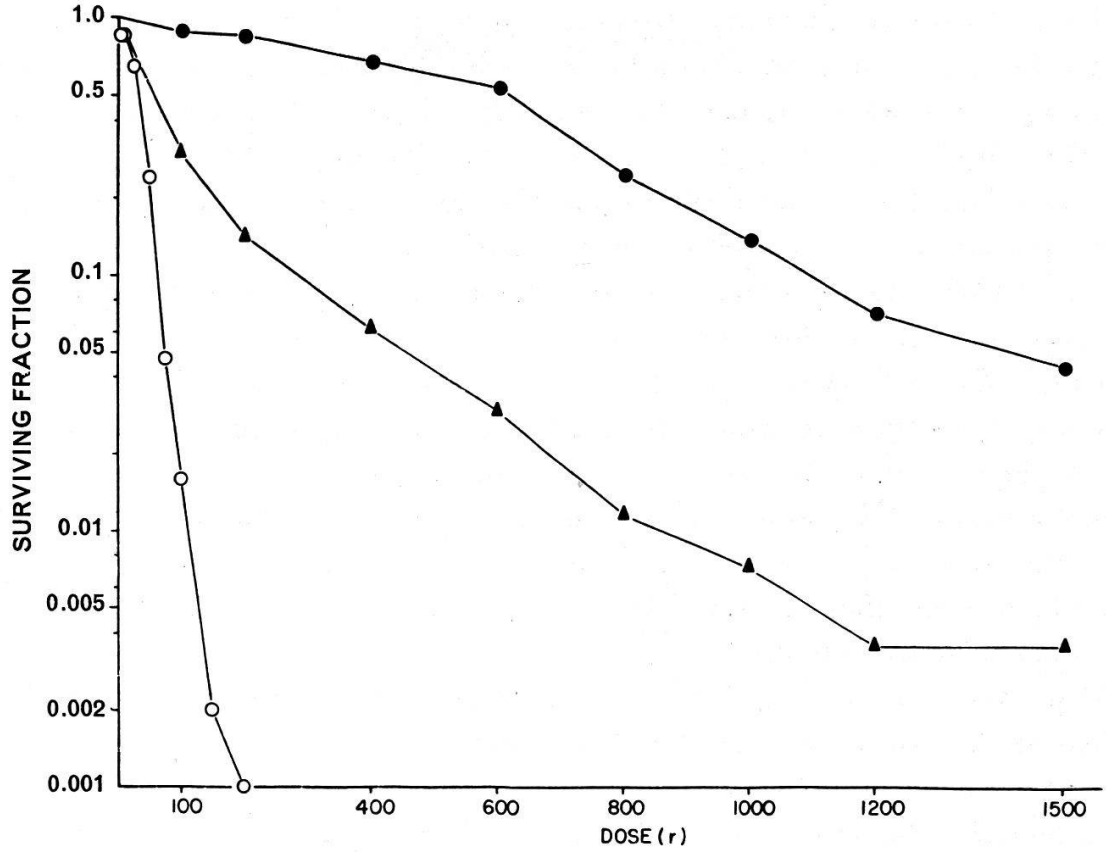

Fig. 2. Relative sensitivity of type A spermatogonia (triangles), type B spermatogonia (open circles), and spermatocytes (closed circles) in the mouse (from *Oakberg*, 1955b).

goes three divisions. Of these resulting eight cells, one remains as a type A spermatogonium to renew the multiplicative cycle; the other seven differentiate into intermediate spermatogonia. The intermediate cells divide to form fourteen type B (crusty) spermatogonia, and the type B cells divide to form twenty-eight resting primary spermatocytes.

Necrosis is the primary response of spermatogonia damaged by radiation. If this degenerative process is allowed to go to completion, decrease in surviving cells then can be related to dose of radiation. In Figure 2 the relative sensitivities of spermatogonia of type A and type B, and primary spermatocytes to X and gamma rays are compared. Since we are especially interested in low-level radiation effects, I only want to discuss the effect of gamma irradiation on spermatogonia. In Figure 3 the survival of type A, intermediate, and type B spermatogonia is given for doses of 5 to 100 r. The effect of 5 r was readily detectable. At doses of 23 r and less, all three spermatogonial types showed the same response, but at higher doses, the curves for intermediate and type B cells continued to drop while the type A spermatogonia showed proportionately less effects. This heterogeneity of sensitivities within the type A population explains the eventual return to fertility of irradiated

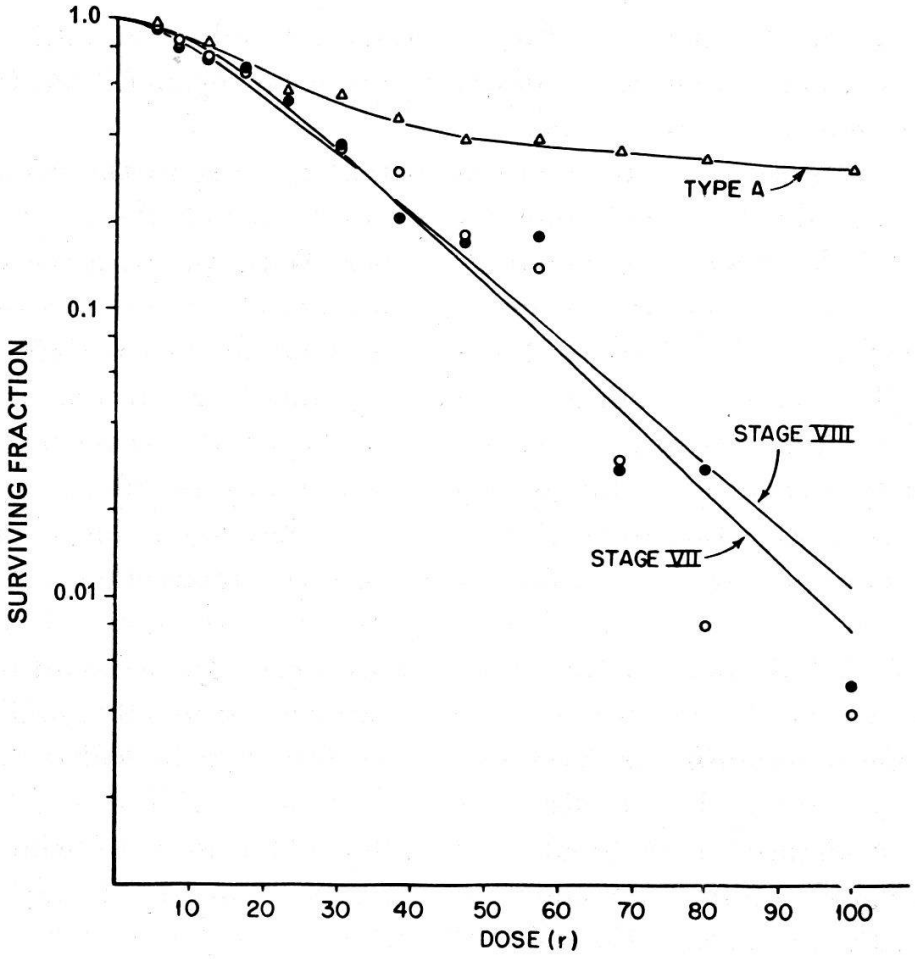

Figure 3. Survival of type A, intermediate (stage VII), and type B (stage VIII) spermatogonia of the mouse after irradiation with Co^{60} gamma rays (from *Oakberg*, 1957).

male mice, since a few of the more resistant cells survive doses of 1000 r or more. Owing to their uniform sensitivities, an LD_{50} of 22.5 ± 1.2 r can be computed for late type A and early intermediate, and an LD_{50} of 21.4 ± 1.3 r for late intermediate and early type B spermatogonia. The response of spermatogonia to low doses has been observed previously (see *Langendorff*, 1936), but recent improvements in concepts of normal spermatogenesis allow accurate identification and quantitative determination of the response of the different cell types.

Mutations have been observed in the early spermatogonial stages after larger amounts of radiation and these have been studied extensively (*Russell*, 1951). Determination of mutation rates in the later, more-sensitive spermatogonial stages is difficult because even relatively low acute doses will kill almost all these more-sensitive spermatogonia. At any rate, since the interval that spermatogenic cells spend as late type A, intermediate, and type B cells is short in relation to the reproductive life span, these cells probably do not constitute a serious hazard in terms of total genetic effects. It is very difficult to sterilize a male by

radiation. First, the sperm itself is very resistant and second, all type A spermatogonia have to be killed in order to prevent regeneration. It may take more than 1500 r to do this.

These results with low doses are important because in the usual discussions it is said that small amounts of radiation, as little as 5 to 25 r, have very little effect on mammalian tissues. With quantitative procedures now available, effects on spermatogonia of the mouse can readily be demonstrated after doses as low as 5 r. I might also mention that these small amounts of radiation, between 5 and 25 r, are not unusual in extensive X-ray treatment or in certain types of X-ray diagnosis. It is well to recognize at this point that there may be effects even at lower levels of radiation in regard to the spermatogonia; however, our present techniques are not sensitive enough to recognize these.

In the female mouse, both direct effects on the ovary and certain genetic effects have been found after very low doses. In the adult ovary, where, in contrast to the testis, no more mitotic germ cells (gonia) are present, the nondividing oocytes have been found to be killed rapidly by a dose of 50 r and probably even lower doses (*Oakberg*, 1958). The sensitive mechanism is of great interest since it cannot be connected with chromosome imbalance and is, furthermore, capable of continuous repair (*L. B. Russell* and *Freeman*, 1958).

A type of genetic effect that can be demonstrated in females by low doses even in small-scale experiments is the production of dominant lethals. The special complications of dominant lethal studies in the mammalian female have been analyzed, and it has been shown that the living embryo per corpus luteum ratios of irradiated and control females accurately measure dominant lethal induction whereas litter size does not (*Russell* and *Russell*, 1955). By this measure, it has been found (Table 1) that dominant lethal induction is very much higher at a stage 8 hours before fertilization (at or near meiotic metaphase I) than at earlier stages. Thus, to produce 50% dominant lethals in prophase oocytes requires about 700 r, but the same frequency is induced at meiotic metaphase I by only about 70 r.

A system very sensitive to even low doses of radiation is the mammalian embryo (*Russell* and *Russell*, 1956). If the embryo is irradiated during the first to the sixth day after fertilization, it will, in a high percentage of cases, die early in development; or it may go ahead and form a fairly normal animal. We have here an «all-or-nothing» effect. In contrast, irradiation during the period between the seventh and fourteenth day (6 ½ to 13 ½ in the development of the mouse embryo) causes little prenatal death (see Figure 4). The embryo here is extremely sensi-

Table 1

Percentage of dominant lethals induced in oocytes by irradiation at various intervals prior to fertilization

Mean treatment-to-fertilization interval (hr)	No. corpora lutea	No. living embryos	Per cent dominant lethals
36.5	100	76	23.9
16.1	42	31	18.7
12.6	84	36	52.8
8.5	92(?)*	1	98.8
Controls	119	108	–

* Corpus luteum counts are unreliable when few or no living embryos are present. To compute dominant lethals in this group an average number of corpora lutea equal to controls was assumed.

tive to radiation. As little as 25 to 50 r, or even less radiation, can cause abnormal development. Figure 5 shows the effects of irradiation at 8 ½ days after conception, when several quantitative skeletal characteristics are observed at birth. Table 2 gives the percentage incidences of a great variety of skeletal abnormalities. It should be pointed out that different types of damage have shown different dose effect curves. In time, when enough data have been collected, one may get a fairly good

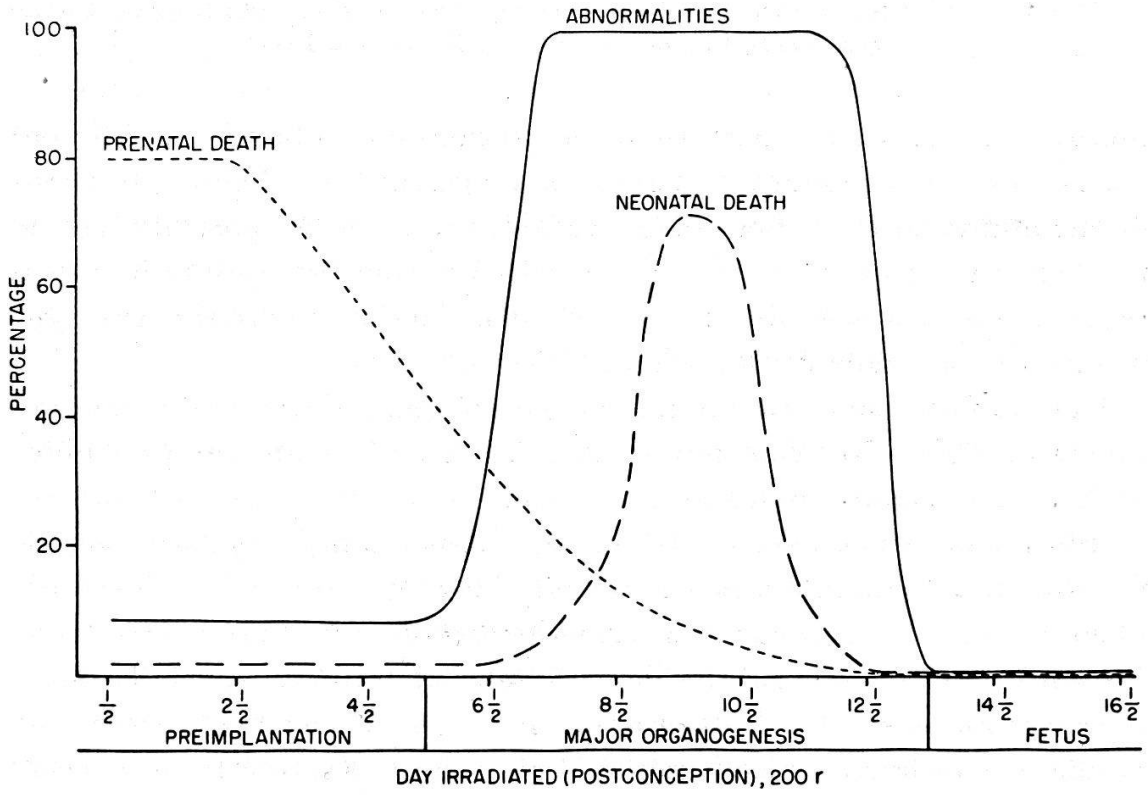

Fig. 4. Incidence of death and abnormalities at term following 200 r at various stages in postnatal development of mice (from *Russell* and *Russell*, 1954).

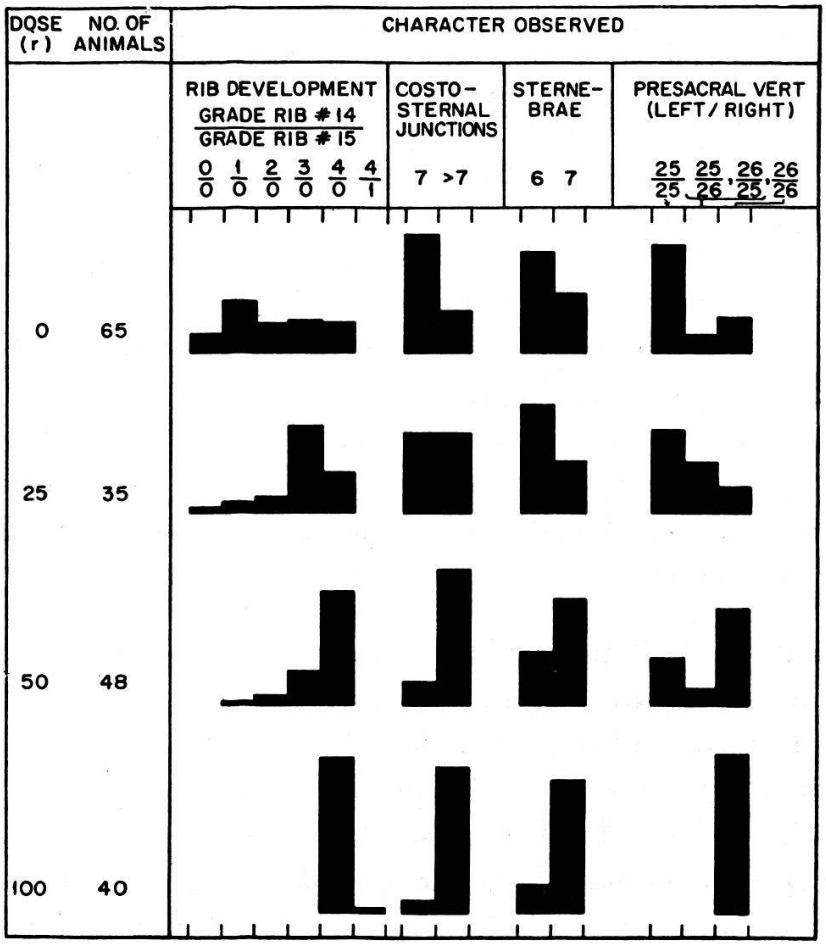

Fig. 5. Effects of irradiation on day 8½ postconception on various quantitative skeletal characters in the mouse (from *L. B. Russell*, 1957).

picture of the entire pattern of malformation induced at different times by different energy values of ionizing radiation. The implications of the ability of these low-energy values, i.e., 25 r and possibly less, to produce effects in embryos is an extremely important one to be considered in medical practice. A recommended procedure to reduce this type of hazard was made by *Russell* and *Russell* (1952).

I would now like to go into a discussion of some of the newer developments in regard to blood groups that seem to indicate the possibility of following somatic mutations in blood groups in man which has formerly not been possible. I refer to the extensive investigations now in progress in several laboratories to study blood groups and the incidence of changes in blood groups that formerly were thought to be quite stable. Very interesting work has developed in our laboratory, especially under the direction of Dr. *K. C. Atwood*, dealing with the effects of recognition of changes in human blood groups. Following is a quote from a recent abstract of *K. C. Atwood* and *S. L. Scheinberg* (1958): «This has in fact been found in the form of a small proportion of exceptional erythrocytes

Table 2

Percentage incidence of abnormalities of the axial skeleton after irradiation of strain Balb/c mouse embryos 7½ days after conception

X-ray dose (r) →	0	25	50	100
No. of animals observed →	39	20	29	6
Abnormalities		Incidence (%)		
Cervical				
Atlas, arcus anterior	0	10.0	13.8	100
Atlas, lateral masses	0	5.0	24.1	100
Axis dyssymphysis	23.1	40.0	48.3	100
Centra, one or more reduced	5.4	21.1	19.2	75
Centra, one or more absent	0	10.5	26.9	100
Centra, "jumbled"	0	0	23.1	66.7
Arches, "jumbled"	0	0	3.4	66.7
Arches, miscellaneous	0	0	3.4	16.7
Thoracic				
Centra, simple split	25.6	40.0	38.0	33.3
Centra, uneven split	5.1	15.0	41.4	100
Centra, "jumbled"	0	0	0	100
Arches, "jumbled"	0	0	0	83.3
Arches, other	0	0	0	16.7
Lumbar				
Centra, split	10.3	20.0	38.0	80
Thorax				
Ribs, fused	0	0	0	100
Costal cartilages, fused	0	0	10.3	100
Sternum, "jumbled"	0	0	17.2	100

lacking A antigen in normal AB and in heterozygous A individuals. The proportion of such cells was determined by an isotope dilution method involving successive agglutinations of Cr^{51}-labeled cells with excess carrier (unlabeled) cells. At age ca. 35, the proportion is about 0.001 indicating a mutation rate of about 3×10^{-9} per hour. The cells were isolated from AB bloods in quantities sufficient for phenotypic characterization. They show a great increase in H substance, are fully agglutinated by anti-B, and sometimes show partial agglutination with anti-A reagents other than the one used in the isolation procedure. A mutational origin is not proved, but the results are highly suggestive.»

Next I would like to discuss some of the newer developments in regard to protection against these radiation damages that I have discussed. I have mentioned that the removal of oxygen increases the resistance of cells, especially grasshopper neuroblast, but this is a general phenomenon wherever living cells are involved. I would like to tell you about some of the new compounds developed recently by *D. G. Doherty* in

our laboratory. These are isothiourea derivatives of cysteamine (β-mercaptoethylamine). One of the best of these contains three carbons in its main chain and has a methyl substituted for a hydrogen in the guanyl group. These compounds are especially successful in that they protect the bone marrow or other tissues where there is intense blood circulation. It is also important to point out that these compounds protect only during the process of irradiation. However, this work has been done only with mice.

Of course the other development that is very interesting is the transplantation of healthy bone marrow in animals to replace bone marrow damaged by radiation. Here very extensive new developments have taken place which would take a long lecture to describe in detail. In any case the developments are of considerable promise, and the new points that have been developed, especially in regard to immunology and longevity, will be discussed by the speaker following me. I just want to say here that chemical compounds have been able to reduce the incidence of leukemia produced in mice by radiation, and you probably know Kaplan (*Kaplan* et al., 1953) has shown that induced leukemia rate can be reduced by transplantation of bone marrow.

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